

Title: *Production of Human Parathyroid Hormone
From Microorganisms*

Inventor(s): Kaare M. GAUTVIK, *et al.*

Appl. No.: 09/668,154

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FIG. 1

DNA SEQUENCE FOR HUMAN
PREPROPARATHYROID HORMONE

10 30 50
ATGATHCCNGCNAARGAYATGGCNAARGTNATGATHGTNATGYTNGCNATHGTGTTTYYTN
70 90 110
ACNAARWSNGAYGGNAARWSNGTNAARAARWSNGTWSNGARATHCARYTNATGCAY
130 150 170
AAYYTNGGNAARCAYYTNAAYWSNATGGARWSNGTNGARTGGYTNGNAARAARYTNCAR
190 210 230
GAYGTNCAYAAYYTGYTNGCNYTNGGNGCNCNYTNGCNCNMGNGAYGCNGGWSNCAR
250 270 290
MGNCCNMGNAARAARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGGNGAR
310 330
GCNGAYAARGCNGAYGTNAAYGTNYTNACNAARGCNAARWSNCARTTR

M = A OR C

R = A OR G

N = A OR T

S = C OR G

Y = C OR T

H = A OR C OR T

N = A OR G OR C OR T

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FIG. 2

DNA SEQUENCE FOR HUMAN
PREPROPARATHYROID HORMONE IN PLASMID pSSHPTH-10

```

      10              30              50
TATGATGATACCTGCAAAAGACATGGCTAAAGTTATGATTGTCATGTTGGCAATTTGTTT

      70              90              110
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAAGAGATCGTGGAGTGAATACAGCTTAT

      130             150             170
GCATAACCTGGGAAAACATCTGAACCTCGATGGAGAGAGTGAATGGCTGCCTAAGAAGCT

      190             210             230
GCAGGATGTGCACAATTTTGTGCCCTTGGAGCTCCTCTAGCTCCAGAGATGCTGTTT

      250             270             290
CCAGAGGCCCCGAAAAAGGAAGACAATGTCTTGTTGAGAGCCATGAAAAAGTCTTGGA

      310             330
GAGGCAGACAAAGCTGATGTGAATGTATTAAGCTAAATCCAGTGA
```

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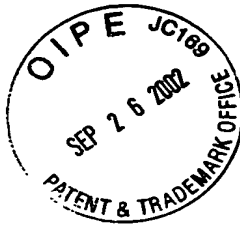


FIG. 3

PORTION OF DNA SEQUENCE OF THE PLASMID
FOR INSERTION INTO E. COLI, CODING FOR HUMAN
PREPROPARATHYROID HORMONE WITH FLANKING SEQUENCES.

10 30 50
TATGATGATHCCNGCNAARGAYATGGCNAARGTNATGATHGTNATGYTNGCNATHGTGTT

70 90 110
YYTNACNAARWSNGAYGGNAARWSNGTNAARAARMGWSNGTNWSNGARATHCARYTNAT

130 150 170
GCAYAAYYTNGGNAARCAYYTNAAYWSNATGGARMGNGTNGARTGGYTNMGNAARAARYT

190 210 230
NCARGAYGTNCAYAAYYTYGTNGCNYTNGGNGCNCNYTNGCNCNMNGAYGCN6GNWS

250 270 290
NCARMGNCNMGNAARAARGARGAYAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGG

310 330 350
NGARGCNGAYAARGCNGAYGTNAAYGTNYTNACNAARGCNAARWSNCARTTRAAATGAAA

370 390 410
ACAGATATTGTGAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATACATGCTGCTAATTC

430
AAAGCTCTATTA

M = A OR C

R = A OR G

W = A OR T

S = C OR T

Y = C OR T

H = A OR C OR T

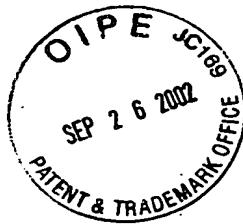
N = A OR G OR C OR T

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FIG. 4

DNA SEQUENCE FOR HUMAN PREPROPARATHYROID
HORMONE IN PLASMID pSSHPTH-10 WITH FLANKING SEQUENCES

```

      10              30              50
TATGATGATACCTGCAAAAGACATGGCTAAAGTTATGATTGTCATGTTGGCAATTTGTTT

      70              90              110
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAGAGATCTGTGAGTGAAATACAGCTTAT

      130             150             170
GCATAACCTGGGAAAACATCTGAACTCGATGGAGAGAGTAGAATGGCTGCGTAAAGAACT

      190             210             230
GCAGGATGTGCACAATTTTGTGCCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTC

      250             270             290
CCAGAGGCCCCGAAAAAAGGAAGACAATGTCTTGGTTGAGAGCCATGAAAAAAGTCTTGG

      310             330             350
AGAGGCAGACAAAGCTGATGTGAATGTATTAAGTAAAGCTAAATCCAGTGAAAAATGAAA

      370             390             410
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATACATGCTGCTAATTC

      430
AAAGCTCTATTA
```



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FIG. 5

DNA SEQUENCE CODING FOR PREPROPARATHYROID HORMONE
IN pSSHPTH-10 WITH FLANKING SEQUENCES, SHOWING
THE CORRESPONDING AMINO ACID SEQUENCE OF
PREPROPARATHYROID HORMONE

```

      10              30              50
TATGATGATACCTGCAAAAGACATGGCTAAAGTTATGATTGTCATGTTGGCAATTTGTTT
MetIleProAlaLysAspMetAlaLysValMetIleValMetLeuAlaIleCysPh

      70              90              110
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAGAGATCTGTGAGTGAAATACAGCTTAT
eLeuThrLysSerAspGlyLysSerValLysLysArgSerValSerGluIleGlnLeuMe

      130             150             170
GCATAACCTGGGAAAACATCTGAACTCGATGGAGAGAGTAGAATGGCTGCGTAAGAAGCT
tHisAsnLeuGlyLysHisLeuAsnSerMetGluArgValGluTrpLeuArgLysLysLe

      190             210             230
GCAGGATGTGCACAATTTTGTGCCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTG6TTC
uGlnAspValHisAsnPheValAlaLeuGlyAlaProLeuAlaProArgAspAlaGlySe

      250             270             290
CCAGAGGCCCCGAAAAAAGGAAGACAATGTCTTGGTTGAGAGCCATGAAAAAAGTCTTGG
rGlnArgProArgLysLysGluAspAsnValLeuValGluSerHisGluLysSerLeuG1

      310             330             350
AGAGGCAGACAAAAGCTGATGTGAATGTATTAAGTAAAGCTAAATCCAGTGAAAAATGAAA
yGluAlaAspLysAlaAspValAsnValLeuThrLysAlaLysSerGlnEnd

      370             390             410
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATACATGCTGCTAATTC

      430
AAAGCTCTATTA
```

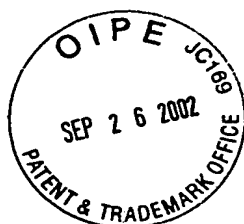


FIG. 6

NUCLEOTIDE SEQUENCE OF THE MF 1-HPTH FISION GENE FROM pS LX5-HPTH1.
NUCLEOTIDE NOS. 1-173 MAKEUP THE MH 1 PROMOTER REGION AND 5'
NONCODING SEQUENCE. 174-440 IS THE MF 1 N-TERMINAL CODING
SEQUENCE. 441-695 IS THE HPTH SEQUENCE OBTAINED FROM pSSHPTH-10.
696-726 IS AN HPTH 3' NONCODING SEQUENCE FROM pSSHPTH-10. 727-732
IS FROM pUC19. 733-874 IS MF 1 3' NONCODING SEQUENCE AND
TRANSCRIPTIONAL TERMINATION SIGNAL.

```

      10              30              50
AGTGAAGAAAAACAAAAAGCAACAACAGGTTTTGGATAAGTACATATATAAGAGGGCCT
      70              90              110
TTTGTTC CATCAAAAATGTTACTGTTCTTACGATTCATTACGATTCAAGAATAGTTCA
      130             150             170
AACAAGAAGATTACAACTATCAATTCATACACAATATAAAGACCAAAAAGAATGAGAT
      190             210             230
TTCCTTCAATTTTTACTGCAGTTTTATTGCGAGCATCCTCCGATTAGCTGCTCCAGTCA
      250             270             290
ACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTCAG
      310             330             350
ATTTAGAAGGGGATTTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGT
      370             390             410
TATTGTTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTTTGG
      430             450             470
ATAAAGAGAGGCTGAAGCTTCTGTGAGTGAAATACAGCTTATGCATAACCTGGGAAAAC
      490             510             530
ATCTGAACTCGATGGAGAGAGTAGAATGGCTGCGTAAGAAGCTGCAGGATGTGCACAATT
      550             570             590
TTGTTGCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTCCCAGAGGCCCCGAAAAA
      610             630             650
AGGAAGACAATGTCTTGTTGAGAGCCATGAAAAAAGTCTTGGAGAGGCAGACAAAGCTG
      670             690             710
ATGTGAATGTATTAATAAGCTAAATCCAGTGAAAATGAAAACAGATATTGTCAGAGT
      730             750             770
TCTGCTCTAGAGTCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATAC
      790             810             830
TTTTCATTTCTCCGTAAACAACCTGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTT
      850             870
CGTTACCAACTTTACACATACTTTATATAGCTAT
```

**FIG. 7****RECEIVED**

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PARTIAL DNA SEQUENCE FOR THE PLASMID FOR INSERTION INTO ~~TEC~~ CENTER 1600/2900
IN WHICH: NUCLEOTIDE NOS. 1-173 MAKEUP THE MF 1 PROMOTER
REGION AND 5' NONCODING SEQUENCE. 174-440 IS THE MF 1 N-TERMINAL
CODING SEQUENCE. 441-695 IS AN HPTH SEQUENCE. 696-726 IS AN
HPTH 3' NONCODING SEQUENCE FROM pSSHPTH-10. 727-732 IF FROM
PUC19. 733-874 IS MF 1 3' NONCODING SEQUENCE AND TRANSCRIPTIONAL
TERMINATION SIGNAL.

```

      10                               30
AGTGAAGAAAACCAAAAAGCAACAACAGGTTTTGGATAAGTACATATATAAGAGGGCCT
      70                               90                               110
TTTGTTCCTCATCAAAAATGTTACTGTTCTTACGATTCATTTACGATTCAAGAATAGTTCA
      130                              150                              170
AACAAGAAGATTACAACTATCAATTTACATACACAATATAACGACCAAAAGAATGAGAT
      190                              210                              230
TTCCTTCAATTTTTACTGCAGTTTTATTGCGAGCATCCTCCGCATTAGCTGCTCCAGTCA
      250                              270                              290
ACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTCAG
      310                              330                              350
ATTTAGAAGGGGATTTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGT
      370                              390                              410
TATTGTTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTTTGG
      430                              450                              470
ATAAAGAGAGGCTGAAGCTWSNGTNWSNGARATHCARYTNATGCAYAAYYTNGGNAARC
      490                              510                              530
AYYTNAAYWSNATGGARMNGTNGARTGGYTNMGNAARAARYTNCARGAYGTNCAYAAYT
      550                              570                              590
TYGTNGCNYTNGGNGCNCNYTNGCNCNMNGAYGCNGGNWSNCARMGNCNMGNAARA
      610                              630                              650
ARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGGNGARGCNGAYAARGCNG
      670                              690                              710
AYGTNAAYGTNYTNACNAARGCNAARWSNCARTRRAAATGAAAACAGATATTGTCAGAGT
      730                              750                              770
TCTGCTCTAGAGTCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATAC
      790                              810                              830
TTTTCATTTCTCCGTAAACAACCTGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTT
      850                              870
CGTTACCAACTTTACACATACTTTATATAGCTAT, WHEREIN
```

M = A OR C

R = A OR G

W = A OR T

S = C OR G

Y = C OR T

H = A OR C OR T

N = A OR G OR C OR T

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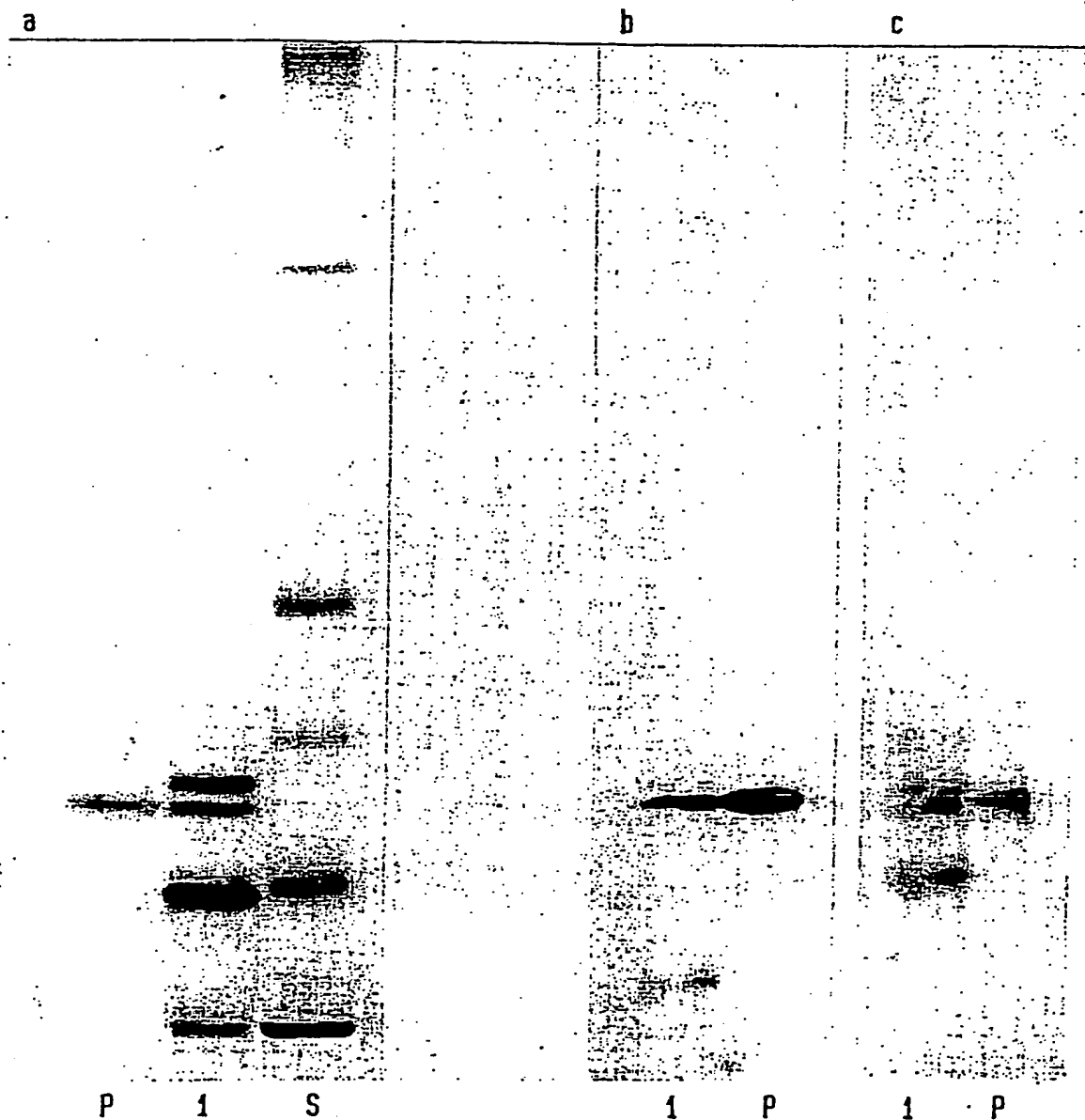
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FIG. 8



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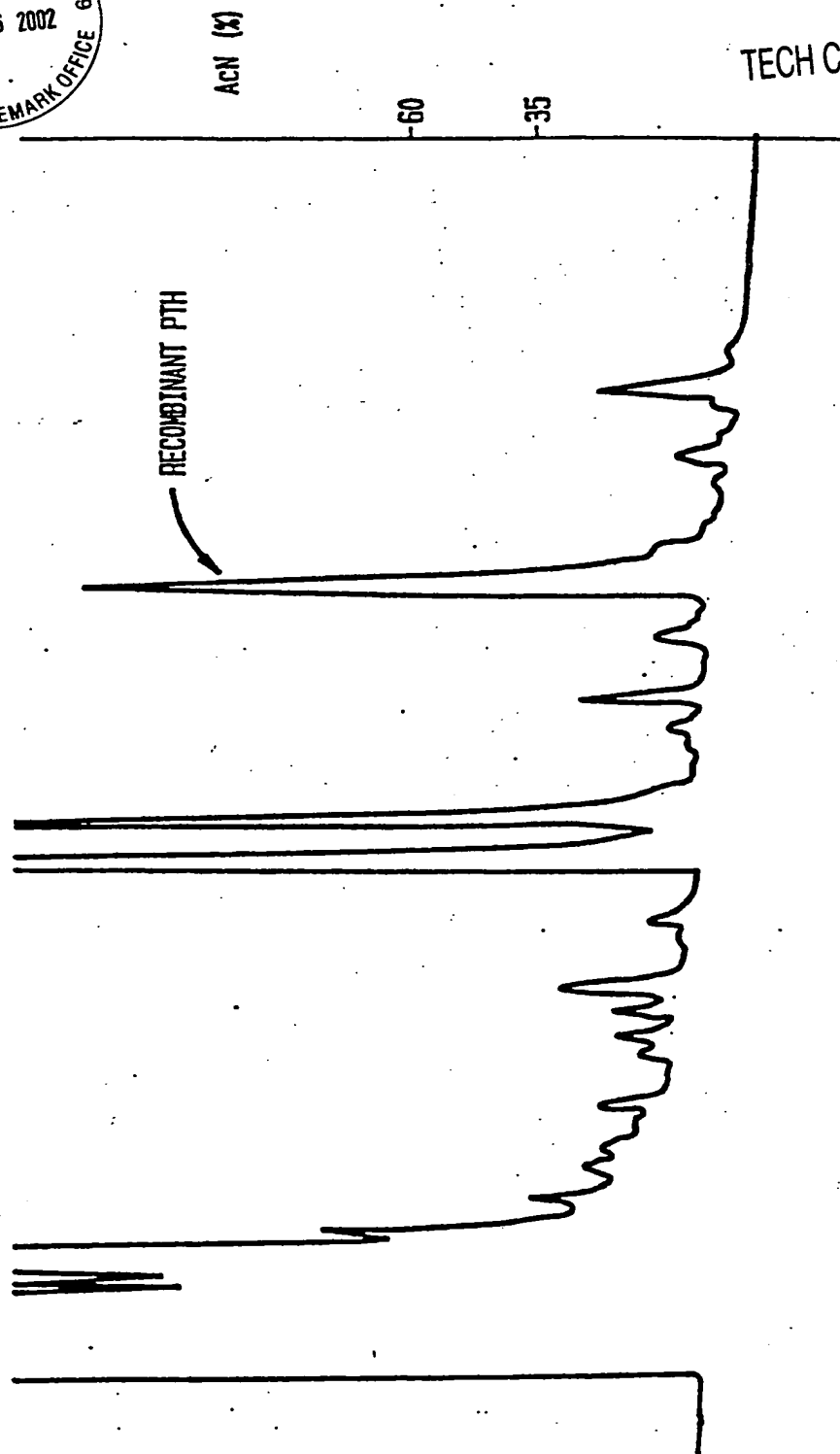
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FIG. 9A



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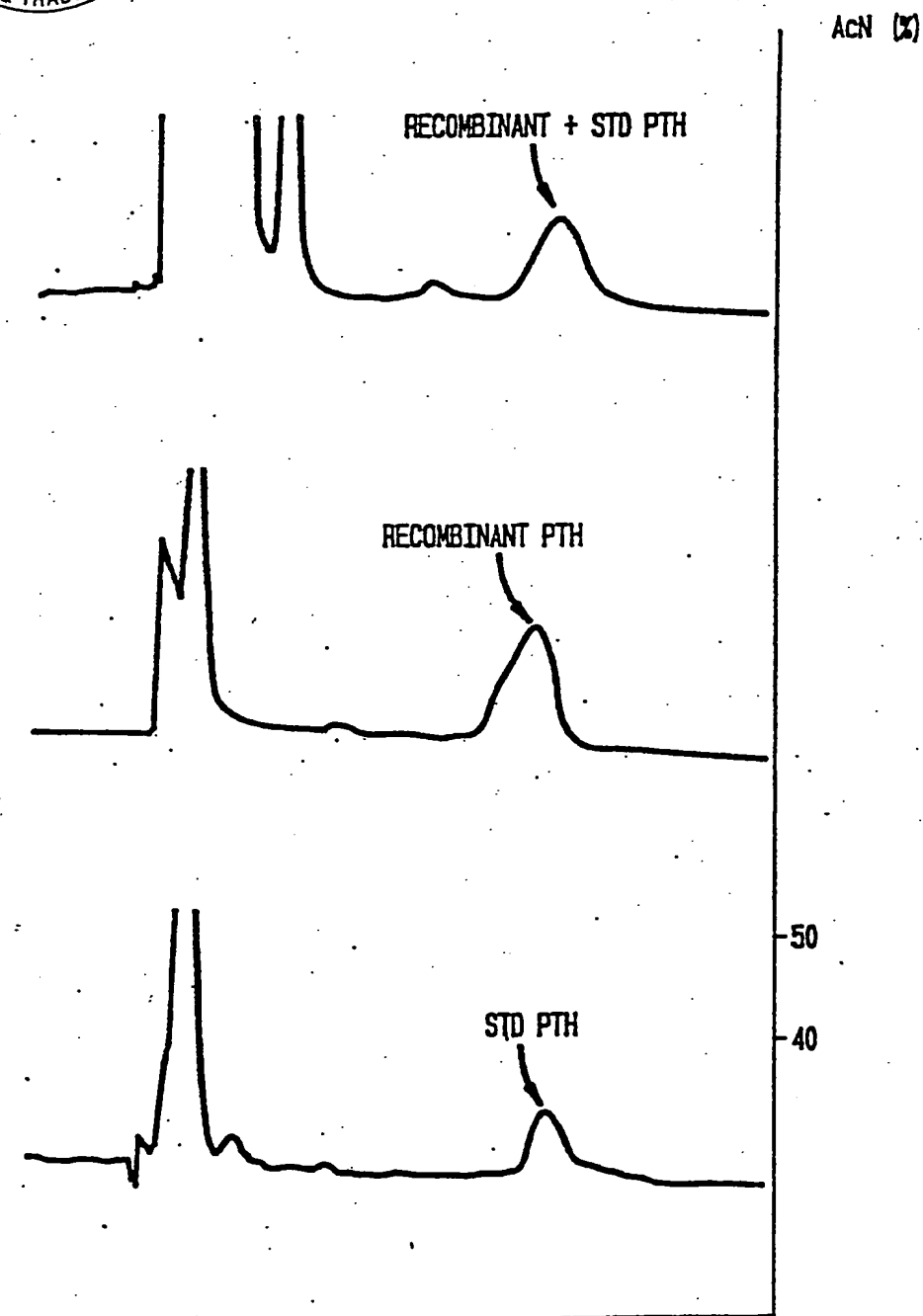
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FIG. 9B

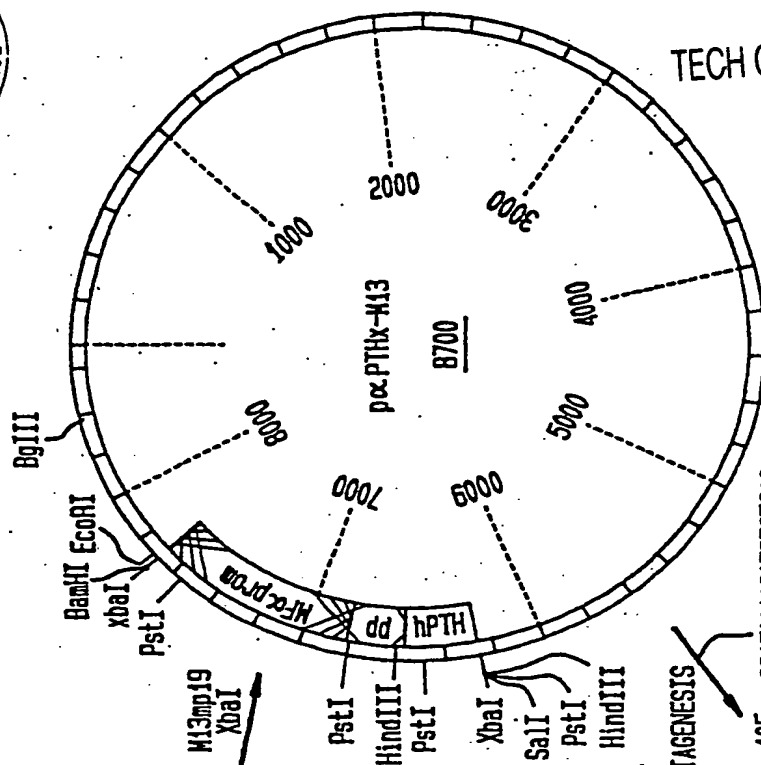


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FIG. 10B



MUTAGENESIS

TO FIG. 10E

FIG. 10A

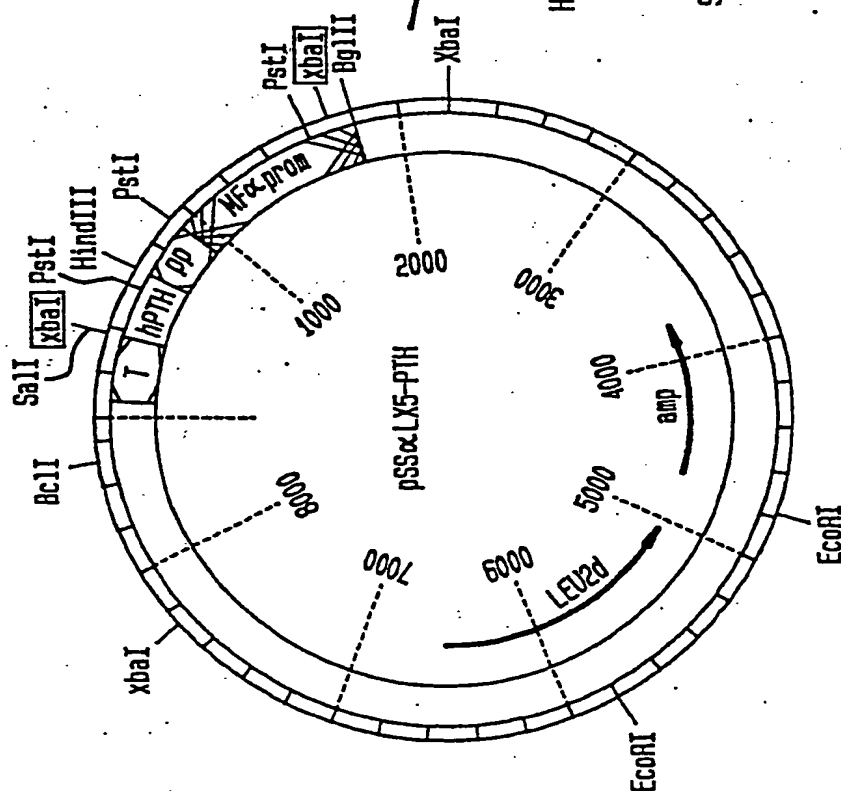




FIG. 10C

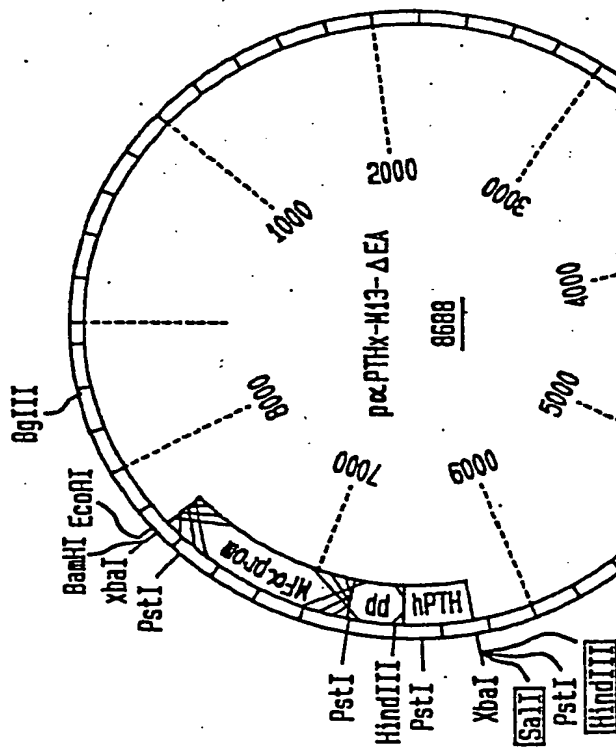
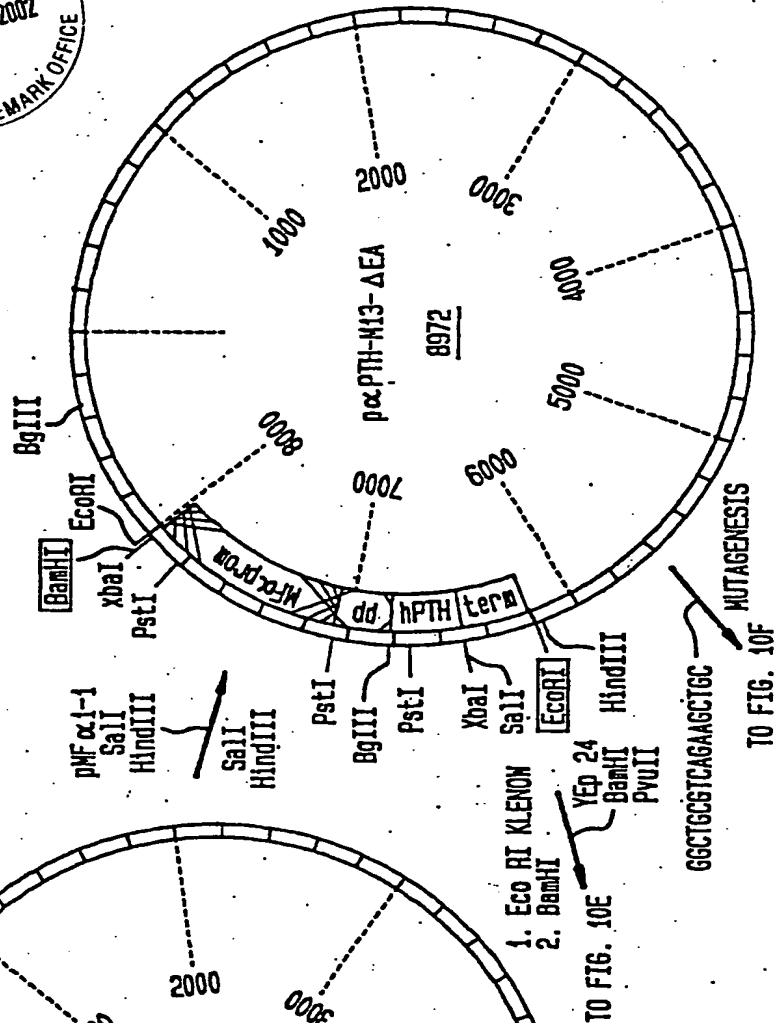


FIG. 10D



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FIG. 10F

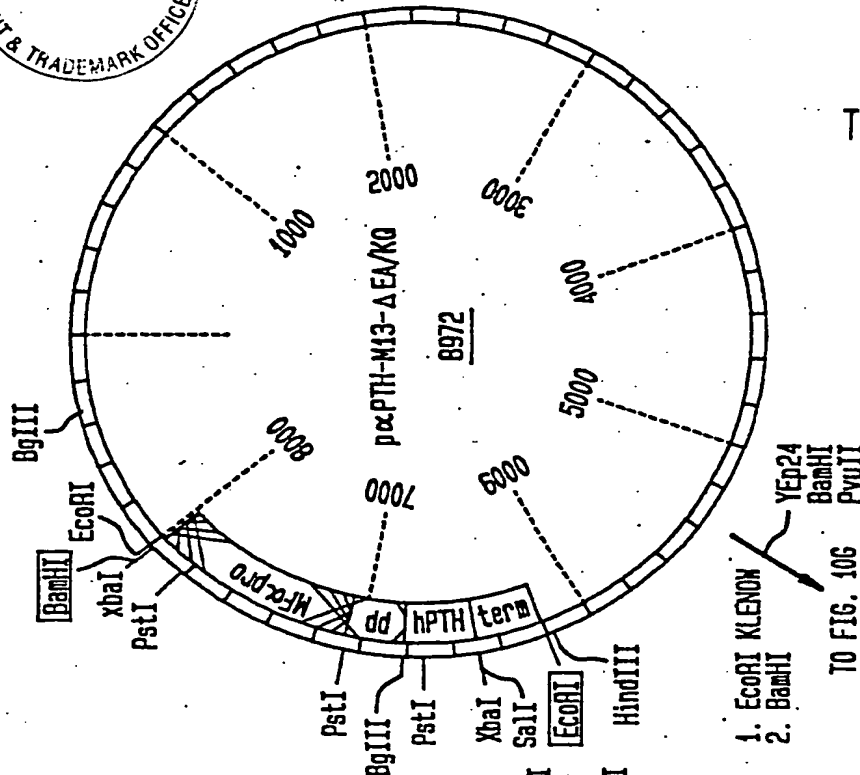
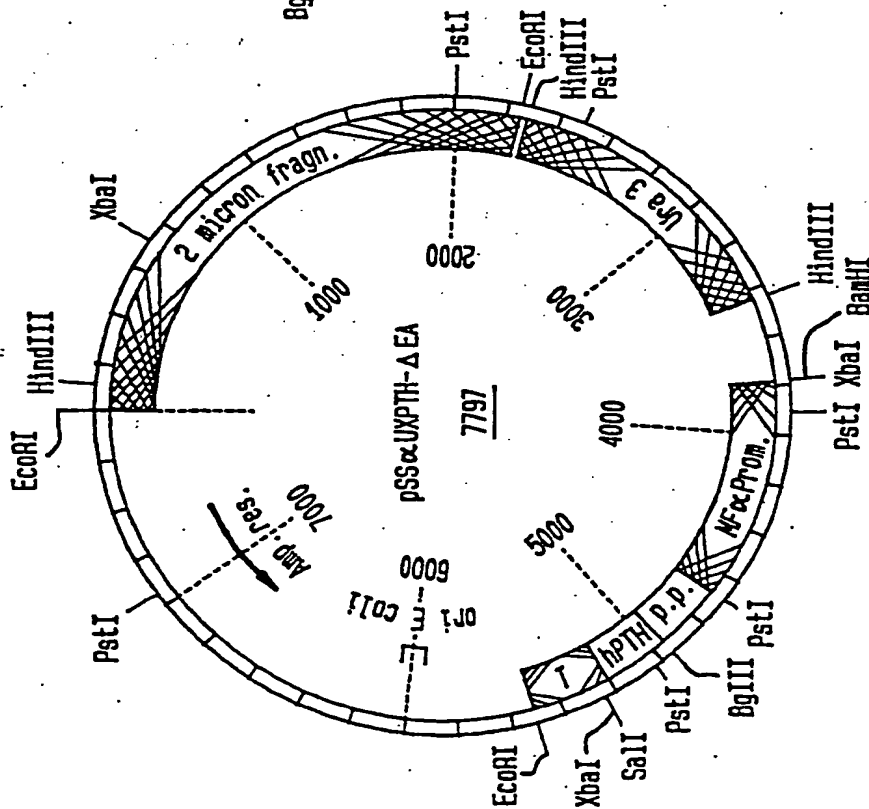


FIG. 10E



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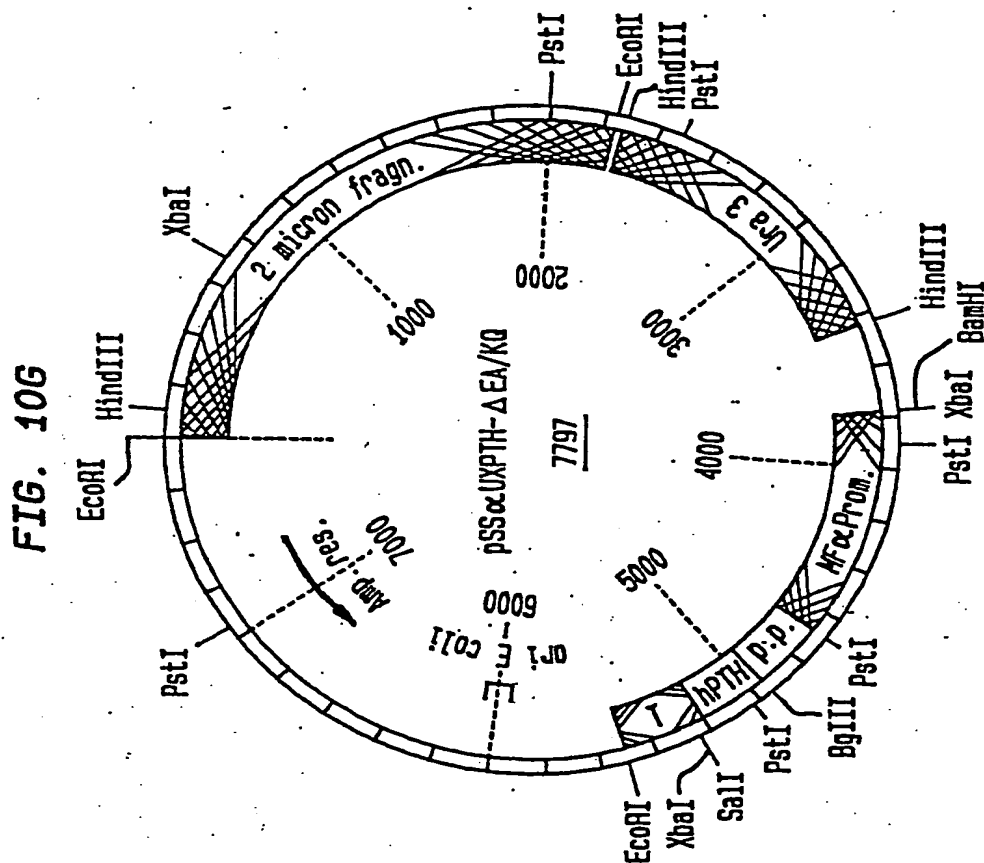
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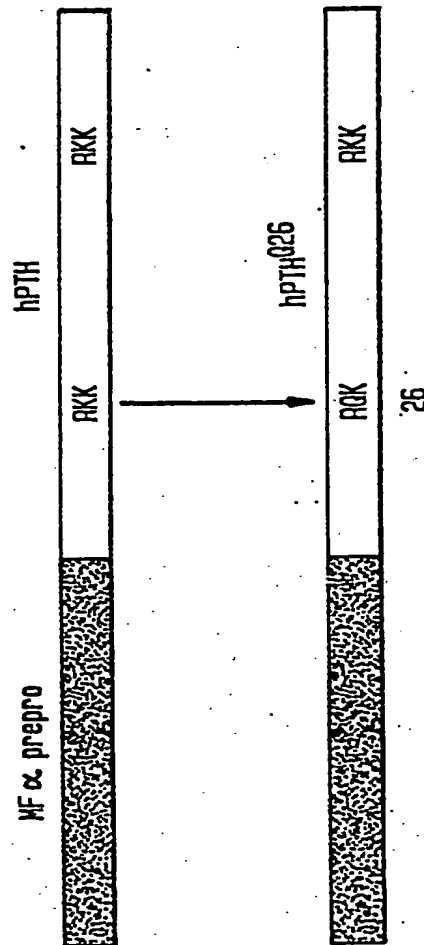
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FIG. 11

IN vitro MUTAGENESIS OF hPTH



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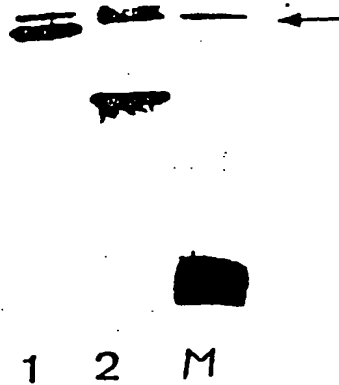


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FIG. 12



Title: *Production of Human Parathyroid Hormone
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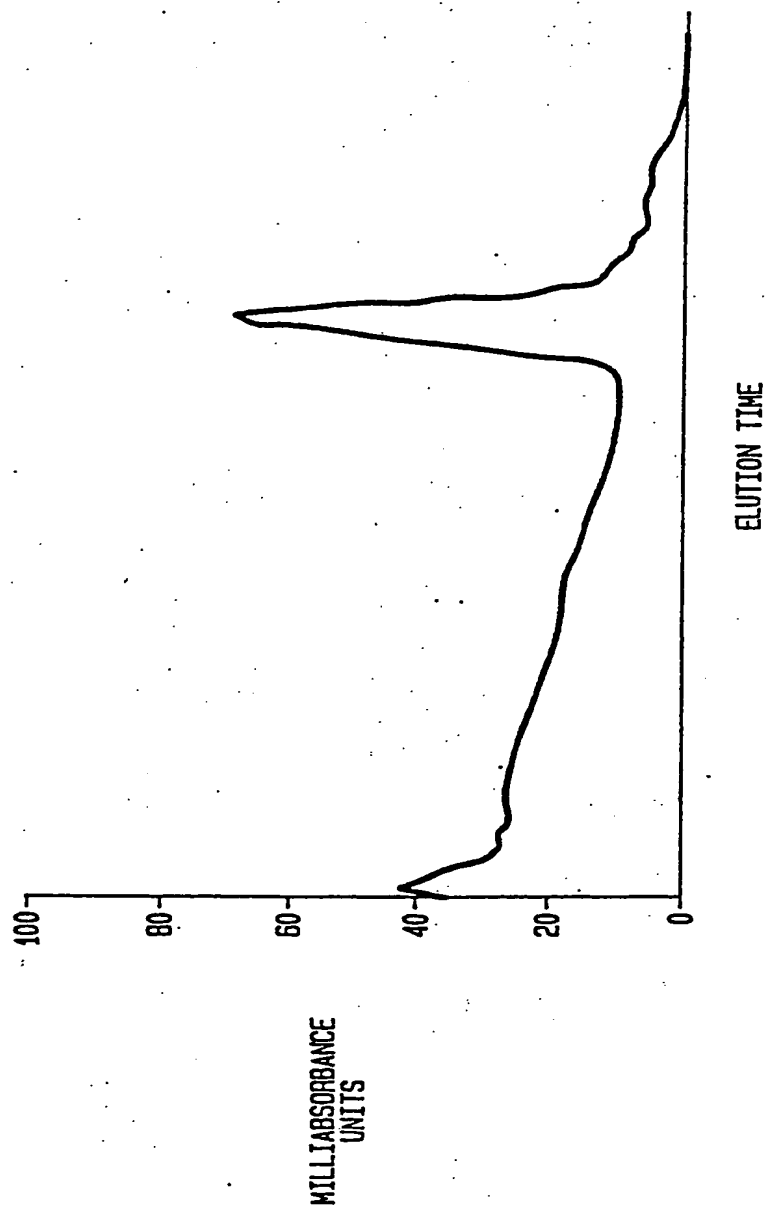
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FIG. 13A

HPLC CHROMATOGRAM OF hPTH (026)



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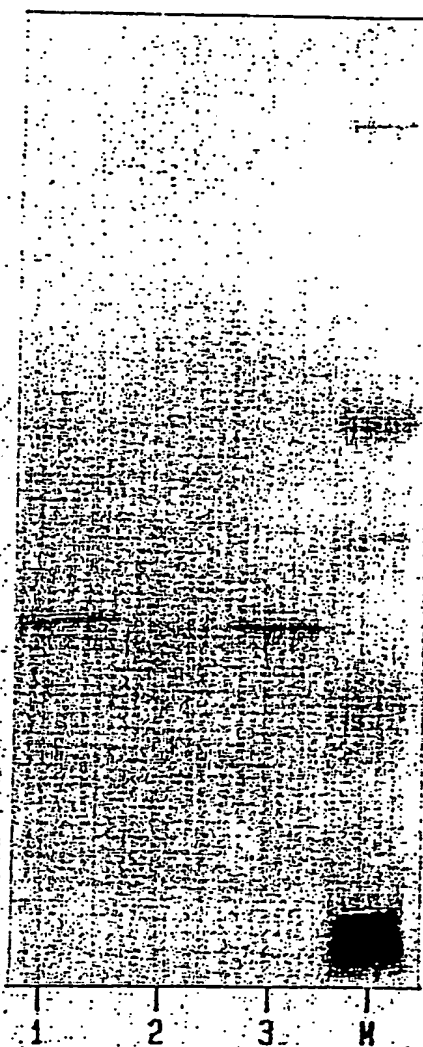


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FIG. 13B



Title: *Production of Human Parathyroid Hormone
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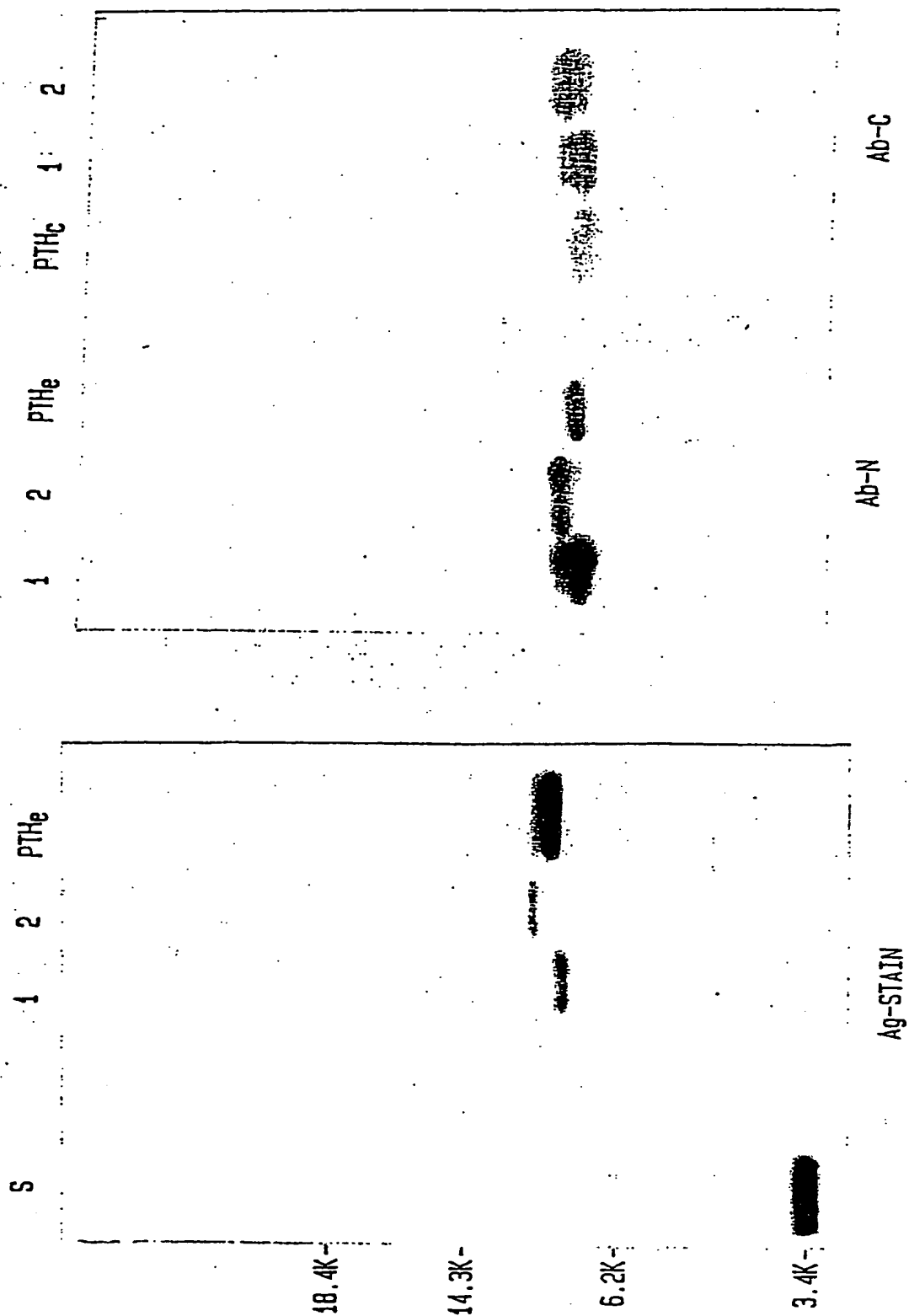
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FIG. 14



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FIG. 15

